

QUANTIFYING PHOTO-TOXICITY IMPACT OF VARIOUS ILLUMINATION MODALITIES USING *C.ELEGANS* EMBRYO.

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Imaging phototoxicity occurs when the illumination of a fluorescently labelled live specimen compromises its viability. It can induce cell shrinkage and death [1], severely damage the mitotic apparatus and causes cell cycle arrest [2]. It is also the source of more subtle artifacts, such as slowed cell dynamics [3] or uncontrolled cell activation [4]. Phototoxicity is a major concern in microscopy, for all these artifacts impair the validity of scientific statements made by imaging.

There have been various attempts to minimize the impact of imaging phototoxicity, either at the sample preparation level [5], by tuning existing illumination modalities [1, 6, 7], or designing new ones [8]. The MEMI project [9] aims at manufacturing a new illumination device for microscopy, based on micro-mirror arrays, that will enable the careful spatiotemporal and angular control of the incident light. The major perk expected from this application for Life-Sciences is a much reduced photo-toxicity through an easy control and design of illumination patterns.

To foster its development for Life-Sciences applications, we derive here a methodology to quantify the phototoxicity impact of a microscopy system. The measurements, based on following the development of a *C.elegans* embryo, yield a quantitative estimate of light dose threshold, above which photo-toxic effects are dominant. This estimate, defined for a given system, can be used to compare different settings on a same setup, or even very different systems, and to state for which the phototoxic impact is the lowest.

We use this protocol to compare thoroughly the main microscopic systems: widefield microscopes, laser scanning confocals, spinning-disc confocals and multi-photon confocals, and derive some counter-intuitive results. They highlight guidelines for the design of experiments with reduced physiological damage. This protocol paves the way towards a Life-science oriented metrology for microscopic setup. It can be used as a non-ambiguous measure to tune a system to achieve the lowest possible phototoxicity and the more physiological conditions.

[1] Hoebe et al., Journal of Microscopy 2008

[2] Dixit & Cyr, Plant Journal 2003

[3] Nishigaki et al., BioTechniques 2006

[4] Saetzler et al., The Journal of Histochemistry and Cytochemistry 1997

[5] Borejdo et al., Journal of Biomedical optics 2008

[6] Ji et al., Nature methods 2008

[7] Devos et al., Cytometry A 2009

[8] Reynaud et al., HSPF Journal 2008

[9] The MEMI project website: <http://memi-fp7.org/>